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# Effects of medicinal plant extracts on growth of *Leishmania (L.)* amazonensis and *Trypanosoma cruzi*

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> This study describes the screening of extracts obtained from 19 species of plants used in Brazilian traditional medicine for treatment of a variety of diseases. The extracts were tested against axenic amastigote and promastigote forms of Leishmania (L.) amazonensis, and epimastigote forms of Trypanosoma cruzi in vitro at a concentration of 100  $\mu$ g/ml. Baccharis trimera, Cymbopogon citratus, Matricaria chamomilla, Mikania glomerata, Ocimum gratissimum, Piper regnellii, Prunus domestica, Psidium guajava, Sambucus canadensis, Stryphnodendron adstringens, Tanacetum parthenium, and Tanacetum vulgare showed significant effects against one or both parasites, with a percentage of growth inhibition between 49.5 and 99%. The extracts showed no cytotoxic effect on sheep erythrocytes. These medicinal plants may be sources of new compounds that are clinically active against L. amazonensis and T. cruzi.

#### Uniterms

- Leishmania (L.) amazonensis
- Trypanosoma cruzi
- Plant extracts
- Pharmacological screening

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#### **INTRODUCTION**

Leishmaniasis is a disease with diverse clinical manifestations that depends on both infecting species of *Leishmania* and the immune response of the host. Several syndromes are subsumed under the term leishmaniasis: most notably visceral, cutaneous and mucosal leishmaniasis (Herwaldt, 1999). The first-choice treatment for the several forms of leishmaniasis is pentavalent antimonials, which are potentially toxic and often ineffective. Second-line compounds used in treatment of unresponsive cases generally include pentamidine and amphotericin B, which may be very toxic (Hepburn *et al.*, 1994; Berman *et al.*, 1996; Croft *et al.*, 1997).

*Trypanosoma cruzi* is the etiological agent of Chagas' disease, which affects 16 to 18 million people in Latin America and is responsible for the death of more than 45,000 patients per year (WHO, 1997). The chronic phase typically occurs 10 to 20 years after the parasite is acquired, and affects 10 to 30% of those infected. It is transmitted to humans by triatomine bugs or through blood transfusion (Viotti *et al.*, 1994). Benznidazole and nifurtimox, the two compounds that have served as the principal antiparasitic drugs for American trypanosomiasis, are not consistently effective and have serious side effects, including cardiac and renal toxicity, and fail to cure most patients with chronic disease (Maya *et al.*, 1997; Veloso *et al.*, 2001). More efficacious drugs are urgently needed to treat patients with leishmaniasis and Chagas' disease.

Although the idea that herbal drugs are totally safe and free from side effects is erroneous, adverse effects of phytotherapeutic agents are less common compared with synthetic drugs. Over the last 15 years, interest in herbal medicines has increased worldwide in both developed and developing countries (Calixto, 2000). Because of the immense flora and cultural aspects the use of plants in the form of crude extracts, infusions or plasters, is a usual practice to treat common infections in Brazil (Calixto, 2000). There is a rich local ethnobotanical bibliography describing the species most frequently used to cure gastrointestinal, respiratory, and urinary problems and skin infections (Corrêa, 1932; Cruz, 1979; Plantas que curam, 1983; Oliveira and Kisue, 1989; Silva and Sant'ana, 1995; Newall et al., 1996; Biazzi, 1996). However, there is still a lack of experimental scientific studies confirming the antibiotic properties of many of these remedies. In vitro antimicrobial screening methods furnish the preliminary observations that are necessary to select, among the crude plant products, those with potentially useful properties for further chemical and pharmacological studies.

The immense chemical diversity and range of bioactivity of plants has led to the development of hundreds of pharmaceutical drugs. Studies in several countries including Brazil, Argentina, Bolivia, Mexico, and Colombia have shown that many plants show activity against *T. cruzi* (Muellas-Serrano *et al.*, 2000; Weniger *et al.*, 2001; Abe *et al.*, 2002; Igweh *et al.*, 2002) and *Leishmania* (Fournet *et al.*, 1992; Torres-Santos *et al.*, 1999; Delorenzi *et al.*, 2001; Ferreira *et al.*, 2002; Salvador *et al.*, 2002).

Extracts derived from plants offer novel possibilities to obtain new compounds that are active against protozoans. We report the results of preliminary screening tests for leishmanicidal and trypanocidal activities of crude extracts from 19 plants used in Brazilian folk medicine for treatment of various diseases: Achillea millefolium, Anacardium occidentale, Baccharis trimera, Cymbopogon citratus, Erythrina speciosa, Eugenia uniflora, Lippia alba, Matricaria chamomilla, Mikania glomerata, Ocimum gratissimum, Piper regnellii, Prunus domestica, Psidium guajava, Punica granatum, Sambucus canadensis, Spilanthes acmella, Stryphnodendron adstringens, Tanacetum parthenium, and Tanacetum vulgare.

#### MATERIAL AND METHODS

#### **Plant material**

The plants were collected in Maringá, State of Paraná in southern Brazil, and identified by comparison with authenticated specimens and the voucher of each species was deposited at the herbarium of the State University of Maringá. Bark of *S. adstringens* was collected in São Jerônimo da Serra, Paraná and a voucher herbarium specimen was deposited in the same herbarium. Aerial parts of *T. parthenium* (Lot 166871) was furnished by Herbarium Laboratório Botânico Ltda.

#### **Preparation of extracts**

The plant parts were dried at room temperature, pulverised, and then macerated in ethanol:water (90:10) for 48 h at 25 °C in the dark. The hydroalcoholic extract thus obtained was evaporated under vacuum and lyophilised, and the residue was directly assayed against the microorganisms.

Bark samples from *S. adstringens* were dried in the dark at room temperature, powdered, and extracted by turbo-extraction in 70% acetone. Next, the crude acetone extract was evaporated under reduced pressure and lyophilised (Mello *et al.*, 1996).

#### Leishmanicidal activity in vitro

For experiments, promastigote forms of the MHOM/ BR/75/Josefa strain of L. amazonensis were inoculated in Warren's medium (brain-heart infusion plus hemin and folic acid) supplemented with 10% of inactivated fetal bovine serum (Gibco Invitrogen Corporation, New York, USA) containing different extracts (100  $\mu$ g/ml), which were added only once to the cultures. Cells were grown in a 24-well plate with each well containing 1 ml of the medium. The starting inocula consisted of protozoans in logarithmic growth phase (1 x 10<sup>6</sup> cells/ml). After 72 h at 28 °C, cell growth was estimated by counting in a haemocytometer (Improved Double Neubauer). All experiments were performed in duplicate, and the results expressed as log number cells/ml and as the percentage of growth inhibition. The extracts tested were dissolved in dimethyl sulfoxide (DMSO), of which the final concentration did not exceed 1% (Yong et al., 2000).

Axenic amastigote cultures, obtained by *in vitro* transformation of infective promastigotes, were maintained in Schneider's insect medium (Sigma Chemical Co., St. Louis, Missouri, USA), pH 4.5, with 20% fetal bovine serum at 32 °C (Ueda-Nakamura, 2001).

#### Trypanocidal activity in vitro

Epimastigote forms of *T. cruzi* Y strain were grown in liver infusion tryptose (LIT) medium supplemented with 10% inactivated fetal bovine serum and assayed with crude extracts (100  $\mu$ g/ml). The extracts were dissolved in 1% DMSO, a concentration which did not affect the growth of the parasites. Next, 1x10<sup>6</sup> protozoa/ml was introduced into 24-well plate each containing 1 ml of diluted extract. Cell growth was determined by counting the parasites with a Neubauer haemocytometer after incubation for 96 h at 28 °C. Assays were performed in duplicate on separate occasions.

#### Red blood cell (RBC) lysis assay

A suspension of freshly defibrinised sheep blood was prepared by adding 2.0 ml of blood to 48.0 ml sterile 5% glucose. Stock solutions of crude extracts solubilized in DMSO were further diluted with sterile 5% glucose to yield final test concentrations of 10 to 1,000  $\mu$ g/ml. One millilitre of RBC suspension was added and gently mixed, and the tubes were incubated at 37 °C. Haemolysis of erythrocytes was indicated by + 25%; ++50%; +++75%; and ++++ 100% lysis. Minimum lytic concentration (MLC) is defined as the lowest concentration of a test compound which produces complete or partial lysis of erythrocytes. These tests were performed in duplicate on separate occasions.

## **RESULTS AND DISCUSSION**

This study demonstrated that crude extracts from several species of plants inhibited the growth of both Leishmania (L.) amazonensis and Trypanosoma cruzi. The 19 plant species evaluated, all traditionally used for treatment of various diseases, are listed in Table I. Numerous extracts of Brazilian medicinal plants have been screened for their antibacterial, antifungal, molluscicidal, antiprotozoal or antiviral activities (Mendes et al., 1999; Alves et al., 2000; Holetz et al., 2002; Bedoya et al., 2002). The ethnobotanical screening tests of crude extracts on growth inhibition of L. amazonensis and T. cruzi are shown in Table II. The hydroalcoholic extracts of the plants C. citratus, M. chamomilla, P. regnellii, T. parthenium, and T. vulgare were the most active against both protozoans, reaching high levels of growth inhibition (over 90%) at 100  $\mu$ g/ml. Previous studies have demonstrated that these plants exhibit antimicrobial and anti-inflammatory activity. The essential oil and sesquiterpene lactones of T. vulgare displayed antibacterial activity (Abad et al., 1995). Holetz et al. (2002) demonstrated antimicrobial activity of extracts of P. regnellii against the gram-positive bacteria Staphylococcus aureus and Bacillus subtilis. Recently, Pessini et al. (2005) isolated 4 neolignans from leaves of the P. regnellii var. pallescens and reported the antifungal activity of the compound conocarpan. Eupomatenoid-7 (neolignan) and fragransin E<sub>1</sub> (lignan) isolated from Aristolochia taliscana immobilized epimastigote forms of T. cruzi (Abe et al., 2002). Extract of M. chamomilla showed anti-inflammatory activity on intact rats inhibiting 41.1% of the paw edema volume. The major constituents of this plant are volatile oils, apigenin, choline umbelliferone, tricontane, phytosterol, anthemic acid, anthemidin azulene, tannin and nicotinic acid (Al-Hindawi et al. 1989). Two hydroxyflavones isolated from leaves and flowers of T. vulgare inhibit the pathways of arachidonate metabolism in mixed peritoneal leucocytes from Wistar rats and reduced inflammation (Williams et al., 1999). Laboratory evidence indicates that T. parthenium extract causes vasodilatation; inhibit phagocytosis, platelet aggregation and secretion of inflammatory mediators (Heptinstall et al., 1985).

Against amastigote forms of L. amazonensis, extracts from three other plants, M. glomerata, O. gratissimum, and P. domestica also gave excellent results, with 97.5, 91.5, and 90.0% growth inhibition, respectively. These plants are antimicrobial and anti-inflammatory agents. An ethanol extract of M. glomerata is effective in inhibiting immunologic inflammation (Fierro et al., 1999), essential oil from O. gratissimum exhibits antibacterial activity in vitro (Nakamura et al., 1999), and extracts from fruits of P. domestica are effective against Staphylococcus aureus and the fungi Scopulariopsis spp. (Stacewicz-Sapuntzakis et al., 2001). Baccharis trimera showed moderate activity against both axenic amastigote and promastigote forms of L. amazonensis and the epimastigote form of T. cruzi, with 64.6, 58.3, and 65.8% growth inhibition, respectively.

Leishmania (L.) amazonensis appeared to be more sensitive than T. cruzi to some of the extracts assayed. Extracts of O. gratissimum, P. domestica, P. guajava, and S. canadensis showed growth inhibition between 52 and 91.5% in L. amazonensis, whereas these extracts were only weakly active against T. cruzi. In preceding studies, Muelas-Serrano et al. (2000) observed that aqueous extracts of B. trimera and P. guajava showed no activity against T. cruzi and Trichomonas vaginalis. On the other hand, S. adstringens showed growth inhibition of 51.9% for T. cruzi, and was weakly active against L. amazonensis, with growth inhibition lower than 36.5%.

Scientific name	Family	Voucher number	Local name	Part used	Use
Achillea . millefolium L	Asteraceae	HUM8424	Mil folhas	Leaf	Flowers and leaves are used to treat wounds, ulcers, diarrhoeae, skin injuries, gastrintestinal disorders, flu, fever and urinary afections <sup>c,d,e</sup>
Anacardium occidentale L.	Anacardiaceae	HUM 10154	Caju	Leaf	This plant is used to treat gastrointestinal, respiratory and skin infection <sup>b</sup>
<i>Baccharis trimera</i> (Less.) DC.	Asteraceae	HUM 10152	Carqueja	Leaf	Leaf are used for treatment of gastrointestinal and respiratory afections, fever and rheumatism <sup>b</sup>
<i>Cymbopogon</i> <i>citratus</i> (DC.) Stapf	Gramineae	HUM 520	Capim limão	Leaf	Used to treat fever and gastrointerstinal diseases <sup>a</sup>
<i>Erythrina speciosa</i> Andrews	Leguminosae Papilionidae	HUM 8416	Eritrina	Stem	The tradicional usage indicates that Erythrina species could have analgesic, anti-inflamatory and antibacterial activiy <sup>d</sup>
Eugenia uniflora L.	Myrtaceae	HUM 8419	Pitanga	Leaf	Leaves are used for treatment of throat complaints, rheumatism, diarrhoeal, inflammatory afections, and helmintic infections <sup>b, e</sup>
<i>Lippia alba</i> (Mill.) N.E.Br.	Verbenaceae	HUM 8421	Erva-cidreira brasileira	Leaf	Its leaves are employed as an infusion or decoction to treat gastrointestinal disorders, dysentery, fever and respiratory disease <sup>e</sup>
Matricaria chamomilla L.	Asteraceae	HUM 10043	Camomila	Flower	Flowers are recommended as anti-inflammatory, carminative, sedative, antipeptic, anti-helmintic and are used for treatment of fever, and skin injuries <sup>b, c</sup>
Mikania glomerata Spreng.	Asteraceae	HUM 8420	Guaco	Leaf	Leaves infusion used as antiseptic, anti-inflamatory and antibacterial <sup>e, f, g</sup>
Ocimum gratissimum L.	Labiatae	HUM 10151	Alfavaca	Leaf	Leaves infusion are used for treatment of upper respiratory tract infections, diarrhea, headache, skin disease, pneumonia, urinary infection, cough and fever <sup>a, b</sup>

**TABLE I** - Traditional use of species selected for antiprotozoan investigation

# TABLE I - cont.

Scientific name	Family	Voucher number	Local name	Part used	Use
<i>Piper regnellii</i> (Miq.) C.DC.	Piperaceae	HUM 8392	Pariparoba	Leaf	Leaf and root are used in the form of crude extracts, infusions or plasters to treat common infections, urinary diseases, respiratoty and pulmonary disorders, leukorrhoeae, gastrointestinal afections and skin infections <sup>b</sup>
Prunus domestica L.	. Rosaceae	HUM 10153	Ameixa	Leaf	Leaves are used to treat some infections specially within the alimentary tract and respiratory disease <sup>b</sup>
Psidium guajava L.	Myrtaceae	HUM 8423	Goiaba	Leaf	Leaf, root and bark extracts are used for treatment of diarrhoeae, leukorrhoeae, cholera, external ulcers and skin diseases °
Punica granatum L.	Puncaceae	HUM 8417	Romã	Fruit	Fruit is recommended as tonic, antispasmodic, antiinflamatory, anti-helmintic, antidiarrhoeal activity and against aphtha <sup>b</sup>
Sambucus canadensis L.	Caprifoliaceae	HUM 8422	Sabugueiro	Leaf	Leaf, flower, and fruit extracts of parts of these plants have been used for respiratoty and pulmonary disorders (cold, coughs, etc.) and antinflamatory topic <sup>c, e, g</sup>
Spilanthes acmella (l.) Murray	Asteraceae	HUM 8418	Jambu	Leaf	A decoction or infusion of leaves and flowers is recommended for stammering, toothache, stomatitis, respiratory and urinary infection and throat complaints <sup>b, e</sup>
<i>Stryphnodendron</i> <i>adstringens</i> (Mart.) Coville	Leguminosae	HUM 3800	Barbatimão	Bark	Bark is used for treatment of leucorrhoea, diarrhea and as anti-inflamatory <sup>g</sup>
<i>Tanacetum</i> <i>parthenium</i> (L.) Sch. Bip.	Asteraceae	L166871	Tanaceto	Aerial Parts	This plant is used against headache, fever, stomachache, toothache, insect bites °
Tanacetum vulgare L.	Asteraceae	HUM 8425	Erva dos Vermes	Leaf	Leaves, flowers and seeds are recommended as anti-inflammatory, carminative, helminth infections and colic <sup>a, c</sup>

<sup>a</sup> Corrêa, M. P., 1932; <sup>b</sup> Plantas que curam, 1983; <sup>c</sup>Newall et al., 1996; <sup>d</sup> Cruz, C. N., 1979; <sup>e</sup> Silva I.; Sant'ana, D. M. G., 1995; <sup>f</sup> Oliveira, P.; Kisue, G., 1989; <sup>g</sup> Biazzi, E. S., 1996.

	% Growth inhibition				
	Leishmania (L.	Trypanosoma cruz			
	Promastigote	Amastigote	Epimastigote		
Achillea millefolium L.	$11.6 \pm 0.3$	$48.2 \pm 6.3$	$39.8 \pm 4.5$		
Anacardium occidentale	$5.4 \pm 7.2$	$32.3 \pm 10.4$	$9.4 \pm 0.5$		
Baccharis trimera (Less) D.C.	$58.3 \pm 8.2$	$64.6 \pm 8.4$	$65.8 \pm 1.1$		
Cymbopogon citratus	$98.0 \pm 0.5$	$95.2 \pm 5.6$	$79.5 \pm 6.4$		
Erythrina speciosa Andrew	$5.1 \pm 3.6$	$28.4 \pm 8.0$	$26.0 \pm 2.8$		
Eugenia uniflora L.	$38.0 \pm 3.8$	$51.6 \pm 0.8$	$34.3 \pm 4.6$		
<i>Lippia alba</i> (Mill.) N.E.Br	$8.5 \pm 5.9$	$57.5 \pm 8.3$	$36.5 \pm 0.7$		
Matricaria chamomilla	$98.1 \pm 0.6$	$92.7 \pm 2.0$	$95.0\pm2.8$		
Mikania glomerata Spreng	$52.5 \pm 11.2$	$97.5 \pm 2.6$	$49.5 \pm 3.5$		
Ocimum gratissimum	$54.7 \pm 0.6$	$91.5 \pm 2.0$	$28.3 \pm 4.2$		
Piper regnellii Miq.	$98.2 \pm 0.5$	$96.8 \pm 3.7$	$89.7 \pm 6.7$		
Prunus domestica	$42.2 \pm 5.0$	$90.0 \pm 7.1$	$5.9 \pm 5.2$		
Psidium guajava L.	$65.4 \pm 5.4$	$52.0 \pm 2.1$	$15.6 \pm 4.4$		
Punica granatum L.	$69.1 \pm 4.4$	$9.1 \pm 3.0$	$11.2 \pm 1.8$		
Sambucus canadensis L.	$65.7 \pm 6.4$	$54.9 \pm 0.6$	$13.8 \pm 6.8$		
Spilanthes acmella Mart.	$4.3 \pm 0.8$	$18.1 \pm 7.1$	$9.3 \pm 4.6$		
Stryphnodendron adstringens Mart	$36.5 \pm 0.5$	$21.0 \pm 0.6$	$51.9 \pm 0.2$		
Tanacetum parthenium	$96.5 \pm 0.2$	$94.3 \pm 4.0$	$93.0 \pm 1.4$		
Tanacetum vulgare L.	$96.4 \pm 3.4$	$99.0 \pm 1.0$	$95.5 \pm 3.5$		

**TABLE II** - Effect of plant extracts on the growth of *Leishmania (L.) amazonensis* and *Trypanosoma cruzi* 

Values represent the mean  $\pm$  S.D. of duplicate determination

Herzog-Soares *et al.* (2002) demonstrated that an ethanol extract of stem bark from *S. adstringens* decreased the number of trypomastigote forms of *T. cruzi* in the blood of mice treated with this extract.

Hydroalcoholic extracts of A. millefolium, A. occidentale, E. speciosa, E. uniflora, L. alba, P. granatum, and S. acmella were inactive against both L. amazonensis and T. cruzi, at 100  $\mu$ g/ml. An ethyl-acetate extract of E. uniflora had the highest activity against both a drug-sensitive and a multi-drug-resistant clone of Trypanosoma congolense (Adewunmi et al., 2001). The medium containing 1% DMSO, negative control, did not affect the growth of the protozoa. Amphotericin B was used as positive control against L. amazonensis and showed 90% of growth inhibition at concentration of 0.116  $\mu$ g/ml. The positive control against T. cruzi was Benznidazole at 10  $\mu$ g/ml that showed 80% of growth inhibition.

Natural products such as alkaloids, terpenes, quinones, and polyphenols have shown potent growth inhibition of *T. cruzi* or *Leishmania brasiliensis* (Wright and Phillipson, 1990). Some Nigerian medicinal plants have been screened for trypanocidal properties (Adewunmi et al., 2001). Their extracts showed good activity against *Trypanosoma brucei* and *Trypanosoma congolense*, suggesting that they might be a potential source of new and selective agents for the treatment of diseases caused by these protozoans. Recently, Weniger *et al.* (2001) reported on antiprotozoal activity of Colombian plants against several strains of *Plasmodium falciparum*, *Leishmania* sp. and *T. cruzi*.

An important criterion in the search for compounds active against *L. amazonensis* and *T. cruzi* with therapeutic potential, is to determine whether they show toxic effects on mammalian host cells. For this purpose, a test of cytotoxicity to sheep erythrocytes was performed in order to determine the ratio of selectivity to biological activity. The haemolytic effects of the crude extracts on sheep blood are shown in Table III. Except *B. trimera*, none of the crude extracts showed any haemolytic effect at 100  $\mu$ g/ml after 60 minutes of incubation.

We suggest that the biological efficacy at this concentration is not due to *in vitro* cytotoxicity. *B. trimera, M. glomerata,* and *T. parthenium* showed no more than 25% lysis after 120 min of incubation. However, the extracts of *A. occidentale, B. trimera, C. citratus, L. alba, M. chamomilla, M. glomerata, O. gratissimum, P. regnellii,* and *T. parthenium* showed haemolytic activity

	Incubation Time (min)						
		60			120		
	Concentration µg/ml						
	1000	500	100	1000	500	100	
Achillea millefolium	0	0	0	0	0	0	
Anacardium occidentale	0	0	0	++	++	0	
Baccharis trimera	++++	++	+	++++	+++	+	
Cymbopogon cytratus	0	0	0	++	0	0	
Erythrina speciosa	0	0	0	+	0	0	
Eugenia uniflora	0	0	0	+	0	0	
Lippia alba	++++	+++	0	++++	+++	0	
Matricaria chamomilla	0	0	0	+++	0	0	
Mikania glomerata	+	0	0	+++	++	+	
Ocimum gratissimum	+	0	0	+++	0	0	
Piper regnellii	0	0	0	+++	+	0	
Prunus domestica	0	0	0	0	0	0	
Psidium guajava	0	0	0	+	0	0	
Punica grantum	0	0	0	0	0	0	
Sambucus canadensis	0	0	0	+++	0	0	
Spilanthes acmella	0	0	0	+++	0	0	
Stryphnodendron adstringens	0	0	0	0	0	0	
Tanacetum parthenium	+++	+++	0	+++	+++	+	
Tanacetum vulgare	0	0	0	++	+	0	
Amphotericin B*	++++	++++	+++	++++	++++	+++	

#### TABLE III - Haemolytic properties of 19 crude extracts and amphotericin B

++++ = 100% lysis; +++ = 75% lysis; ++ = 50% lysis; + = 25% lysis; 0 = no lysis

Minimum lytic concentration at 60 min = 25 mg/ml; 120 min = 12.5 mg/ml

at concentrations of more than 500  $\mu$ g/ml. As reported by Lee *et al.* (1999), amphotericin B showed strong haemolytic activity with 50% lysis at 25  $\mu$ g/ml within 60 min of incubation. The control with Triton X-114 showed a strong haemolytic effect with 100% lysis after 60 min, while 1% DMSO did not cause lysis.

# CONCLUSION

The results of the present study confirmed that the use of medicinal plants in folk medicine contributes significantly to primary health care, and that natural products are potential sources of new and selective agents for the treatment of important tropical diseases caused by protozoans. Further laboratory and clinical studies of these plants are required in order to understand their antiprotozoal principles. Therefore, the most promising extracts were prioritised for further phytochemical analysis on the isolation and the identification of the active compounds with antiprotozoal activity, a work that is already under way.

#### **RESUMO**

## Efeito de extratos de plantas medicinais no crescimento de *Leishmania (L.) amazonensis* e *Trypanosoma cruzi*

Este estudo descreve a triagem de extratos obtidos de 19 espécies de plantas usadas na medicina tradicional brasileira para o tratamento de várias doenças. Os extratos foram testados contra formas amastigota axênica e promastigota de Leishmania (L.) amazonensis, e formas epimastigota de Trypanosoma cruzi in vitro na concentração de 100 µg/ml. Baccharis trimera, Cymbopogon citratus, Matricaria chamomilla, Mikania glomerata, Ocimum gratissimum, Piper regnellii, Prunus domestica, Psidium guajava, Sambucus canadensis, Stryphnodendron adstringens, Tanacetum parthenium, e Tanacetum vulgare apresentaram efeito significante contra um ou ambos parasitas, com a porcentagem de inibição de crescimento entre 49,5 e 99%. Os extratos não mostraram efeito citotóxico em hemácias de carneiro. Essas plantas medicinais podem ser fontes alternativas de novos compostos clinicamente ativos contra L. amazonensis e T. cruzi.

UNITERMOS: Leishmania (L.) amazonensis. Trypanosoma cruzi. Extratos de plantas. Triagem farmacológica.

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# REFERENCES

- ABAD, J.M.; BERMEJO, P.; VILLAR, A. An approach to the genus *Tanacetum* L. (Compositae): phytochemical and pharmacological review. *Phytother: Res.*, London, v. 9, p. 79-92, 1995.
- ABE, F.; NAGAFUJI, S.; YAMAUCHI, T.; OKABE, H.; MAKI, J.; HIGO, H.; AKAHANE, H.; AGULAR, A.; JIMENEZ-ESTRADA, M.; REYES-CHILPA, R. Trypanocidal constituents in plants 1. Evaluation of some mexican plants for their trypanocidal activity and active constituents in guaco, roots of *Aristolochia taliscana*. *Biol. Pharm. Bull.*, Tokyo, v. 25, p. 1188-1191, 2002.
- ADEWUNMI, C.O.; AGBEDAHUNSI, J.M.; ADEBAJO,
  A.C.; ALADESANMI, A.J.; MURPHY, N.; WANDO,
  J. Ethno-veterinary medicine: screening of Nigerian medicinal plants for trypanocidal properties. J. Ethnopharmacol., Limerick, v. 77, p. 19-24, 2001.
- AL-HINDAWI, M.K.; AL-DEEN, I.H.S.; NABI, M.H.A.; ISMAIL, M.A. Anti-inflammatory activity of some Iraqi plants using intact rats. *J. Ethnopharmacol.*, Limerick, v. 26, p. 163-168, 1989.

- ALVES, T.M.A.; SILVA, A.F.; BRANDÃO, M.; GRANDI, T.S.M.; SMÂNIA, E.F.; SMÂNIA JR A.; ZANI, C.L. Biological screening of Brazilian medicinal plants. *Mem. Inst. Oswaldo Cruz*, Rio de Janeiro, v. 95, p. 367-373, 2000.
- BEDOYA, L.M.; SANCHES-PALOMINO, S.; ABAD, M.J.; BERMEJO, P.; ALCAMI, J. Screening of selected plant extract for *in vitro* inhibitory activity on human immunodeficiency virus. *Phytother: Res.*, London v. 16, p. 550-554, 2002.
- BERMAN, J.D. Treatment of New World cutaneous and mucosal leishmaniasis. *Clin. Dermatol.*, v. 14, p. 519-522, 1996.
- BIAZZI, E.S. Saúde pelas plantas. Tatuí: Casa Publicadora Brasileira, 1996. p. 176.
- CALIXTO, J.B. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Braz. J. Med. Biol. Res.*, Ribeirão Preto, v. 33, p. 179-189, 2000.
- CORRÊA, M.P. *Dicionário das plantas úteis do Brasil*, Rio de Janeiro: IBDF. 1932, v.I p. 577, v.II pp. 72-74.
- CROFT, S.L.; URBINA, J.A.; BRUN, R. Chemotherapy of human leishmaniasis and trypanosomiasis. In HIDE, G.; MOTTRAN, J.C.; COOMBS, G.H.; HOLMES, P.H. (Eds.). *Trypanosomiasis and leishmaniasis*. London: CAB International, United Kingdom, 1997. p. 245-247.
- CRUZ, C.N. *Dicionário de plantas úteis no Brasil*. Rio de Janeiro: Civilização Brasileira: 1979. p. 599.
- DELORENZI, J.C.; ATTIAS, M.; GATTASS, C.R.; ANDRADE, M.; REZENDE, C.; PINTO, A.C.; HENRIQUES, A.T.; BOU-HABIB, D.C.; SARAIVA, E.M.B. Antileishmanial activity of indol alkaloid from *Peschiera australis*. *Antimicrob. Agents Chemother.*, Bethesda, v. 45, p. 1349-1354, 2001.
- FERREIRA, M.E.; ARIAS, A.R.; ORTIZ, S.T.; INCHAUSTI, A.; NAKAYAMA, H.; THOUVENEL, C.; HOCQUEMILLER, R.; FOURNET, A. Leishmanicidal activity of two canthin-6-one alkaloids, two major constituents of *Zanthoxylum chiloperone* var. *angustifolium. J. Ethnopharmacol.*, Limerick, v. 80, p. 199-202, 2002.

- FIERRO, I.M.; SILVA, A.C.B.; LOPES, C.S.; MOURA, R.S.; BARJA-FIDALGO, C. Studies on the anti-allergic activity of *Mikania glomerata*. J. Ethnopharmacol., Limerick, v. 66, p. 19-24, 1999.
- FOURNET, A.; ANGELO, A.; MUÑOZ, V.; ROBLOT, F.; HOCQUEMILLER, R.; CAVÉ, A. Biological and chemical studies of *Pera benensis*, a Bolivian plant used in folk medicine as a treatment of cutaneous leishmaniasis. *J. Ethnopharmacol.*, Limerick, v. 37, p. 159-164, 1992.
- HEPBURN, N.C.; SIDDIQUE, I.; HOWIE, A.F.; BECKETT, GJ.; HAYES, P.C. Hepatotoxicity of sodium stibogluconate therapy of American cutaneous leishmaniasis. *Trans. R. Soc. Trop. Med. Hyg.*, London, v. 88, p. 453-455, 1994.
- HEPTINSTALL S.; WHITE A.; WILLIAMSON L.; MITCHELL J.R. Extracts of feverfew inhibit granule secretion in blood platelets and polymorphonuclear leucocytes. *Lancet*, London, v. 1, p. 1071-1074, 1985.
- HERWALDT, B.L. Leishmaniasis. *Lancet*, London, v. 354, p. 1191-1199, 1999.
- HERZOG-SOARES, J. D'ARC; ALVES, R.K.; ISAC, E.;
  BEZERRA, J.C.B.; GOMES, M.H.; SANTOS, S.C.;
  FERRI, P.H. Atividade tripanocida *in vivo* de Stryphnodendron adstringens (Barbatimão verdadeiro)
  e Caryocar brasiliensis (pequi). Rev. Bras. Farmacogn., São Paulo, v. 12, suppl, p. 1-2, 2002.
- HOLETZ, F.B.; PESSINI, G.L.; SANCHES, N.R.; CORTEZ, D.A.G.; NAKAMURA, C.V.; DIAS FILHO, B.P. Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. *Mem. Inst. Oswaldo Cruz*, Rio de Janeiro, v. 97, p. 1027-1031, 2002.
- IGWEH, A.C.; AGUIYI, J.C.; OKWUASABA, F.K. Antitrypanosomal effects of the aqueous extract of *Brassica oleracea*. *Fitoterapia*, Milan, v. 73, p. 17-21, 2002.
- LEE, S.H.; LEE, J.R.; LUNDE, C.S.; KUBO, I. *In vitro* antifungal susceptibilities of *Candida albicans* and other fungal pathogens to polygodial, a sesquiterpene dialdehyde. *Planta Med.*, Stuttgart, v. 65, p. 204-208, 1999.

- MAYA, J.D.; REPETTO, Y.; AGOSIN, M.; OJEDA, J.M.; TLLEZ, R.; GAULE, C.; MORELLO, A. Effects of nifurtimox and benznidazole upon glutatione and trypanothione content in epimastigote, trypomastigote and amastigote forms of *Trypanosoma cruzi*. *Mol. Biochem. Parasitol.*, Amsterdam, v. 86, p. 101-106, 1997.
- MELLO, J.C.P.; PETEREIT, F.; NAHRSTEDT, A. Flavan-3-ols and Prodelphinidins from *Stryphnodendron adstringens*. *Phytochemistry*, New York, v. 41, p. 807, 1996.
- MENDES, N.M.; QUEIROZ, R.O.; GRANDI, T.S.M.; ANJOS, A.M.G.; OLIVEIRA, A.B.; ZANI, C.L. Screening of Asteraceae (Compositae) plant extracts for molluscicidal activity. *Mem. Inst. Oswaldo Cruz*, Rio de Janeiro, v. 94, p. 411-412, 1999.
- MUELLAS-SERRANO, S.; NOGAL, J.J.; MARTÍNEZ-DÍAZ, R.A.; ESCARIO J.A.; MARTÍNEZ-FERNÁNDEZ, A.R.; GOMEZ-BARRIO, A. *In vitro* screening of American plants extracts on *Trypanosoma cruzi* and *Trichomonas vaginalis*. *J. Ethnopharmacol.*, Limerick, v. 71, p. 101-107, 2000.
- NAKAMURA, C.V.; UEDA-NAKAMURA, T.; BANDO, E.; MELO, A.F.N.; CORTEZ, D.A.G.; DIAS FILHO, B.P. Antibacterial activity of *Ocimum gratissimum* L. essencial oil. *Mem. Inst. Oswaldo Cruz*, Rio de Janeiro, v. 94, p. 675-678, 1999.
- NEWALL, C.A.; ANDERSON, L.A.; PHILLIPSON, J.D. Plantas medicinais: guia para profissional de saúde. São Paulo: Premier, 1996. p. 59-248.
- OLIVEIRA, P.; KISUE, G. Fundamentos de Farmacobotânica. Rio de Janeiro: Ateneu, 1989. 412 p.
- Plantas que curam a natureza a serviço de sua saúde. In ALZREGARAY D.; ALZREGARAY C. (Eds.). São Paulo: Três Livros e Fascículos, 1983. v. I IV.
- PESSINI, G.L.; DIAS FILHO, B.P.; NAKAMURA, C.V.; CORTEZ, D.A.G. Antifungal activities of the extracts and neolignans from *Piper regnellii* (Miq.) C. DC. var. *pallescens* (C. DC.) Yunck. J. Braz. Chem. Soc., Campinas, in press, 2005.

- SALVADOR, M.J.; FERREIRA, E.O.; PRAL, E.M.F.; ALFIERI, S.C.; ALBUQUERQUE, S.; ITO, I.Y.; DIAS, D.A. Bioactivity of crude extracts and some sonstituents of *Blutaparon portulacoides* (Amaranthaceae). *Phytomedicine*, Stuttgart, v. 9, p. 566-571, 2002.
- SILVA I.; SANT'ANA, D.M.G. Noções sobre o organismo humano e a utilização de plantas medicinais. Cascavel: Assoeste Editora Educativa, 1995. p. 203.
- STACEWICZ-SAPUNTZAKIS, M.; BOWEN, P.E.; HUSSAIN, E.A.; DAMAYANTI-WOOD, B.I.; FARNSWORTH, N.R. Chemical composition and potential health effects of prunes: a functional food? *Crit. Rev. Food Sci. Nutr.*, Boca Raton, v. 41, p. 251-286, 2001.
- TORRES-SANTOS, E.C.; MOREIRA, D.L.; KAPLAN, M.A.C.; MEIRELLES, M.N.; ROSSI-BERGMANN,
  B. Selective effect of 2',6'-dihydroxy-4'methoxychalcone isolated from *Piper aduncum* on *Leishmania amazonensis*. *Antimicrob. Agents Chemother.*, Bethesda, v. 43, p. 1234-1241, 1999.
- UEDA-NAKAMURA, T.; ATTIAS, M.; DE SOUZA, W. Megasome biogenesis in *Leishmania amazonensis*: a morphometric and cytochemical study. *Parasitol. Res.*, Würzburg, v. 87, p. 89-97, 2001.
- VELOSO, V.M.; CARNEIRO, C.M.; TOLEDO, M.J.O.; CHIARI, E.; TAFURI, W.L.; BAHIA, M.T. Variation in susceptibility to benznidazole in isolates derived from *Trypanosoma cruzi* parenteral strains. *Mem. Inst. Oswaldo Cruz*, Rio de Janeiro, v. 96, p. 1005-1011, 2001.

- VIOTTI, R.; VIGLIANO, C.; ARMENTI, H.; SEGURA, E. Treatment of chronic Chagas' disease with benznidazole: clinical and serological evolution of patients with long term follow-up. *Am. Heart J.*, St. Louis, v. 127, p. 151-162, 1994.
- WENINGER, B.; ROBLEDO, S.; ARANGO, G.J.; DEHARO, E.; ARAGÓN, R.; MUÑOZ, V.; CALLAPA, J.; LOBSTEIN, A.; ANTON, R. Antiprotozoal activities of Colombian plants. J. Ethnopharmacol., Limerick, v. 78, p. 193-200, 2001.
- WHO World Health Organization. Chagas disease. Thirtheenth Programme Report UNDP/WB/TDR, Geneve, p. 112-23, 1997.
- WILLIAMS, C.A.; HARBONE, J.B.; GEIGER, H.; HOULT, J.R.S. The flavonoids of *Tanacetum parthenium* and *T. vulgare* and their anti-inflammatory properties. *Phytochemistry*, New York, v. 51, p. 417-423, 1999.
- WRIGHT, C.W.; PHILLIPSON, J.D. Natural products and the development of selective antiprotozoal drugs. *Phytother. Res.*, London, v. 4, p.127-139, 1990.
- YONG, V.; SCHMITZ, V.; VANNIER-SANTOS, M.A.; LIMA, A.P.C.A.; LALMANACH, G.; JULIANO, L.; GAUTHIER, F.; SCHARFSTEIN, J. Altered expression of cruzipain and a cathepsin B-like target in a *Trypanosoma cruzi* cell line displaying resistance to synthetic inhibitors of cysteine-proteinases. *Mol. Biochem. Parasitol.*, Amsterdam, v. 109, p. 47-59, 2000.
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